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# *In Vitro* Studies in Sunflower (*Helianthus annuus* L.)

**V. Anitha*, Farzana Jabeen and N. A. Ansari**

Department of Genetics & Plant Breeding, College of Agriculture, ANGRAU, Hyderabad, Andhra Pradesh, India

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**Correspondence to**

**E-mail*: sathishpindi@gmail.com

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**Abstract**

Tissue culture studies were carried out in the Tissue Culture Laboratory, College of Agriculture, ANGRAU, Rajendranagar, Hyderabad, India in the year 2006 to determine the effects of different hormonal concentrations and combinations on explants (hypocotyl, cotyledonary leaf and primary leaf) for callus induction and regeneration in five sunflower genotypes, viz. APSH-11, NDSH-1, DRSF-108, BRISF-73 and EC-68415. Among the five sunflower cultivars, NDSH-1 responded positively for both callus induction and rhizogenesis, while DRSF-108 was better for caulogenesis. In different hormonal combinations, MS medium supplemented with NAA + BA proved better for callus induction in all explants than 2,4-D + kinetin. Among the 10 treatments studied 0.5 mg $l^{-1}$ BA showed maximum caulogenesis.

## 1. Introduction

Sunflower (*Helianthus annuus* L.) is one of the most important oil seed crops in the world. Biotechnological methods are used in complementing and circumventing the limitation of conventional methods for increasing the crop productivity. These non-conventional tools could be of great value in tailoring crop plants as desired. Tissue culture studies were conducted in sunflower to determine the response of different explants and varieties and the nutritional requirements of various explants for callus induction and growth. The tissue culture studies have been mostly confined to optimize the culture conditions for whole plantlet regeneration since the culture of callus tissue provides an alternative technique for regeneration of whole plant. Depending on the sucrose concentration in culture medium (Jeannin et al., 1995), somatic embryos or shoots can be induced in *in vitro* conditions from immature zygotic embryos of sunflower. Depending on agar concentration sucrose concentration and carbohydrate source had significant effect on callusing in sunflower (Saji and Sujatha, 1998). A medium containing 3% sucrose induces only shoots while somatic embryos are formed in a medium containing 12% sucrose. Callus induction and plant organogenesis varied depending upon genotype, explant and medium used. APSH-11 hybrid showed highest callus induction on MS medium (MS + 0.5 mg BA $l^{-1}$ + 0.5 mg NAA $l^{-1}$) (Amit et al., 2001).

## 2. Materials and Methods

Experiments were carried out with five sunflower genotypes, viz. APSH-11, NDSH-1, DRSF-108, BRISF-73 and EC-68415 in the Tissue Culture Research Laboratory of Department of Genetics and Plant Breeding, College of Agriculture, ANGRAU, Rajendranagar, Hyderabad, India during 2005-06. 100 mg powder of NAA or 2,4-D was dissolved in 2-5 ml of 1 N sodium hydroxide (NaOH). After complete dissolution, the final volume was made to 100 ml by adding distilled water. From that solution 1ml was taken into 1 l of basal media.100 mg powder of BA or kinetin was dissolved in 2-5 ml 1 N hydrochloric acid (HCl). After complete dissolution, the final volume was made to 100 ml by adding distilled water. From that solution 1ml was taken into 1 l of basal media. Seeds were sterilized with 0.1% mercuric chloride ($HgCl_2$) for 5 m and then rinsed with sterile distilled water for three times of 5 m each to remove any traces of mercuric chloride ($HgCl_2$).The seeds were then transferred to the filter paper boats of sterile culture tubes. Concentrations and combination of different hormones in Murashige and Skoog (MS) medium were as $T_1$=MS + NAA (1.0) + BA (0.5) (mg $l^{-1}$), $T_2$=MS + NAA (0.5) + BA (2.0) (mg $l^{-1}$), $T_3$=MS + NAA (1.0) + BA (1.0) (mg $l^{-1}$), $T_4$=MS + 2,4-D (1.0) + Kinetin (0.5) (mg $l^{-1}$), $T_5$=MS + 2,4-D (1.0) +

Kinetin (1.0) (mg $l^{-1}$), $T_6$=MS + 2,4-D (2.0) + Kinetin (1.0) (mg $l^{-1}$), $T_7$=MS + BA (0.5) (mg $l^{-1}$), $T_8$=MS + BA (1.0) (mg $l^{-1}$), $T_9$=MS + BA (2.0) (mg $l^{-1}$) and $T_{10}$=MS + Kinetin (0.5) (mg $l^{-1}$). $T_1$ to $T_6$ treatments were used for callus induction studies, and $T_7$ to $T_{10}$ treatments were used for regeneration purpose. The result thus obtained was subjected to statistical analysis using Complete Randomized Design. Seven days old explants (hypocotyl, cotyledonary leaf and primary leaf) were excised from old aseptic seedlings and inoculated on MS medium. It was observed that the hypocotyl expanded in length and width, while cotyledonary and primary leaves showed downward curling in all the treatments. After 3-4 days of inoculation, callus was initiated from the cut surfaces of these explants. After plating, it takes 14 days for the initiation of the callus in different concentrations of various hormones. The observations recorded based on the effect of MS basal medium in combination with different hormones on callusing from various explants was studied given below:

$$\text{Callus induction (\%)} = \frac{\text{Number of cultures with callus}}{\text{Total number of cultures inoculated}} \times 100$$

The percentage of morphogenesis was calculated using the following formulae:

$$\text{Shoot differentiation (\%)} = \frac{\text{Number of calli that regenerated shoots}}{\text{Total number of calli inoculated}} \times 100$$

$$\text{Rooting (\%)} = \frac{\text{Number of calli that regenerated roots}}{\text{Total number of calli inoculated}} \times 100$$

## 3. Results and Discussion

### *3.1. Callus induction*

Callus induction frequency was recorded at the end of the 4th week after inoculation of different explants (Table 1). Among the five selected sunflower genotypes, NDSH-1 recorded highest mean frequency (91.39%) of callus induction followed by DRSF-108 (85.85%) and lowest was with EC-68415 (74.09%). It may be due to the differences in genetic constitution of genotype. Among six media combinations tried, MS medium supplemented with 1 mg $l^{-1}$ NAA + 1 mg $l^{-1}$ BA ($T_3$) recorded the highest callus induction (93.78%) followed by 1 mg $l^{-1}$ 2,4-D + 1 mg $l^{-1}$ kinetin ($T_5$) (82.28%). Among the hormonal combinations, NAA + BAP treatment showed early callus initiation compared to 2,4-D + kinetin treatment. This result is in agreement with that of Lupi et al. (1987) reported early callus induction from hypocotyl and cotyledon explants of cv. Argentario on MS medium supplemented with NAA and BA. However, MS medium supplemented with 2 mg $l^{-1}$ 2,4-D and

Table 1: Callus induction frequency from various explants [hypocotyl (H), cotyledonary leaf (CL) and primary leaf (PL)] in different hormonal treatments

| Treatment | APSH-11 | | | | NDSH-1 | | | | DRSF-108 | | | | BRISF-73 | | | | EC-68415 | | | | Total mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | H | CL | PL | Mean | H | CL | PL | Mean | H | CL | PL | Mean | H | CL | PL | Mean | H | CL | PL | Mean | |
| $T_1$ | 80.30 | 84.90 | 94.40 | 86.53 | 84.90 | 78.18 | 88.23 | 83.77 | 86.66 | 84.90 | 76.27 | 82.61 | 67.16 | 92.30 | 93.15 | 84.20 | 58.60 | 68.75 | 77.70 | 68.35 | 81.09 |
| $T_2$ | 67.79 | 90.16 | 75.75 | 77.90 | 100.00 | 93.02 | 86.95 | 93.32 | 69.69 | 76.30 | 89.55 | 78.51 | 82.30 | 77.46 | 81.92 | 80.56 | 74.62 | 76.30 | 67.64 | 72.85 | 80.63 |
| $T_3$ | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 84.74 | 97.50 | 80.35 | 87.53 | 84.90 | 85.90 | 73.36 | 81.39 | 93.78 |
| $T_4$ | 64.28 | 69.69 | 82.25 | 72.07 | 93.65 | 90.62 | 95.77 | 93.35 | 88.05 | 92.30 | 80.35 | 86.90 | 73.70 | 82.14 | 87.17 | 81.00 | 81.94 | 66.10 | 69.49 | 72.51 | 81.17 |
| $T_5$ | 77.77 | 85.96 | 78.18 | 80.64 | 88.23 | 90.76 | 84.78 | 87.92 | 94.40 | 95.77 | 84.74 | 91.64 | 69.23 | 81.30 | 80.00 | 76.84 | 84.40 | 65.40 | 73.30 | 74.37 | 82.28 |
| $T_6$ | 82.35 | 93.20 | 88.23 | 87.93 | 98.59 | 86.56 | 84.90 | 90.02 | 76.27 | 71.60 | 78.46 | 75.44 | 68.40 | 78.90 | 74.50 | 73.93 | 80.30 | 68.42 | 76.56 | 75.09 | 80.48 |
| Mean | 78.74 | 87.31 | 86.46 | 84.17 | 84.22 | 89.85 | 90.10 | 91.39 | 85.84 | 86.81 | 84.89 | 85.85 | 74.25 | 84.93 | 82.84 | 80.67 | 77.46 | 71.81 | 73.00 | 74.09 | 83.23 |
| SEm± | | | | | | | | | | | | | | | | | | | | CD ($p$=0.05) | |
| $F_1$ (1st generation) | | | | | | | | 0.39 | | | | | | | | | | | | 0.77 | |
| $F_2$ (2nd generation) | | | | | | | | 0.36 | | | | | | | | | | | | 0.70 | |
| $F_3$ (3rd generation) | | | | | | | | 0.27 | | | | | | | | | | | | 0.54 | |
| $F_1$ x $F_2$ | | | | | | | | 0.88 | | | | | | | | | | | | 1.72 | |
| $F_1$ x $F_3$ | | | | | | | | 0.68 | | | | | | | | | | | | 1.33 | |
| $F_2$ x $F_3$ | | | | | | | | 0.62 | | | | | | | | | | | | 1.22 | |
| $F_1$ x $F_2$ x $F_3$ | | | | | | | | 1.52 | | | | | | | | | | | | 2.99 | |

1mg $l^{-1}$ kinetin ($T_6$) recorded least callus induction of 80.48%, respectively. The overall response of callusing ability observed in the explants showed a degree of plasticity in all explants for their cultural requirements.

*3.2. Shoot induction*

The media with different concentrations of NAA and BA, the caulogenesis in all five sunflower genotypes mean ranged from 52.63 to 97.82% with an overall mean of 79.48% using hypocotyl as explant (Table 2).

Among the five genotypes tested, NDSH-1 recorded a maximum regeneration frequency of 97.82% at 0.5 mg $l^{-1}$ BA ($T_7$) followed by 89.09% with 1mg $l^{-1}$ NAA and 1 mg $l^{-1}$ BA ($T_8$) and least shoot induction frequency of 75.47% was recorded by 0.5 mg $l^{-1}$ kinetin ($T_{10}$) in hypocotyl explant. MS medium supplemented with kinetin + IAA (1 mg $l^{-1}$ each) produced

Table 2: Morphogenesis frequency from various explants of sunflower genotypes

| Treatment | APSH-11 | | NDSH-1 | | DRSF-108 | | BRISF-73 | | EC-68415 | | Mean | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | SI | RI | SI | RI | SI | RI | SI | RI | SI | RI | SI | RI |
| $T_7$ | 75.75 | 74.70 | 97.82 | 91.50 | 97.70 | 89.30 | 89.09 | 88.10 | 95.52 | 62.50 | 91.17 | 81.82 |
| $T_8$ | 77.77 | 76.00 | 89.09 | 88.70 | 92.85 | 90.50 | 87.67 | 79.90 | 76.36 | 52.30 | 84.74 | 77.48 |
| $T_9$ | 73.68 | - | 81.39 | - | 89.04 | - | 76.08 | - | 66.03 | - | 77.24 | - |
| $T_{10}$ | 63.60 | - | 75.47 | - | 68.18 | - | 64.10 | - | 52.63 | - | 64.79 | - |
| Mean | 72.70 | 75.35 | 85.94 | 90.10 | 86.94 | 89.90 | 79.23 | 84.00 | 72.63 | 57.30 | 79.48 | 79.65 |

| | SEm± | CD ($p$=0.05) |
|---|---|---|
| $F_1$ (1st generation) | 0.39 | 0.77 |
| $F_2$ (2nd generation) | 0.36 | 0.70 |
| $F_3$ (3rd generation) | 0.27 | 0.54 |
| $F_1 \times F_2$ | 0.88 | 1.72 |
| $F_1 \times F_3$ | 0.68 | 1.33 |
| $F_2 \times F_3$ | 0.62 | 1.22 |
| $F_1 \times F_2 \times F_3$ | 1.52 | 2.99 |

SI: Shoot induction; RI: Root induction

direct callusing and rapid shoot elongation within 10 days, on 11th day rhizogenesis from callus (Anwar et al., 2001). In DRSF-108, MS medium supplemented with 0.5 mg $l^{-1}$ BA ($T_7$) recorded highest shoot induction of 97.7% with hypocotyl followed by 92.85% in 1 mg $l^{-1}$ NAA and 1 mg $l^{-1}$ BA ($T_8$) and least shoot induction frequency of 68.18% by MS medium with 0.5 mg $l^{-1}$ kinetin ($T_{10}$). Multiple shoot formation was achieved from the explant hypocotyls both directly and indirectly. These reports are in agreement with Krasnyanski and Menczel (1991). Comparative studies on callus induction and plant regeneration from various explants like hypocotyl, cotyledonary leaf and primary leaf in sunflower were also reported by Bohorova et al. (1990).

*3.3. Root induction*

In media with different concentrations, the rhizogenesis from cotyledonary leaf and hypocotyl of all sunflower genotypes mean ranged from 52.3 to 91.5% with an overall mean value of 79.65% (Table 2). Among the five genotypes tested, NDSH-1 recorded highest (91.5%) root induction followed by DRSF-108 (89.3%) and minimum root induction percentage was recorded in EC-68415 (62.5%) with 0.5 mg $l^{-1}$ BA ($T_7$). Maximum callusing was observed when NAA and BA were used as source of auxin and cytokinin for callus formation and morphogenesis. Maximum callusing was observed when NAA and BA were used as source of auxin and cytokinin for callus formation and morphogenesis (Yanchich and Vilor, 1988) which are similar to the results obtained in the present study. It indicated that influence of hormonal combinations, potential differences in physiological status of the explants and the molecular mechanism underlying regeneration process.

## 4. Conclusion

Among the treatments, MS medium supplemented with 0.5 mg $l^{-1}$ BA was found to be superior to all other combinations in inducing maximum caulogenesis and also rhizogenesis from calli irrespective of the genotype. Among five sunflower genotypes studied, NDSH-1 showed superiority over all other genotypes both for callus induction and rhizogenesis while DRSH-108 was better over all other genotypes for caulogenesis.

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